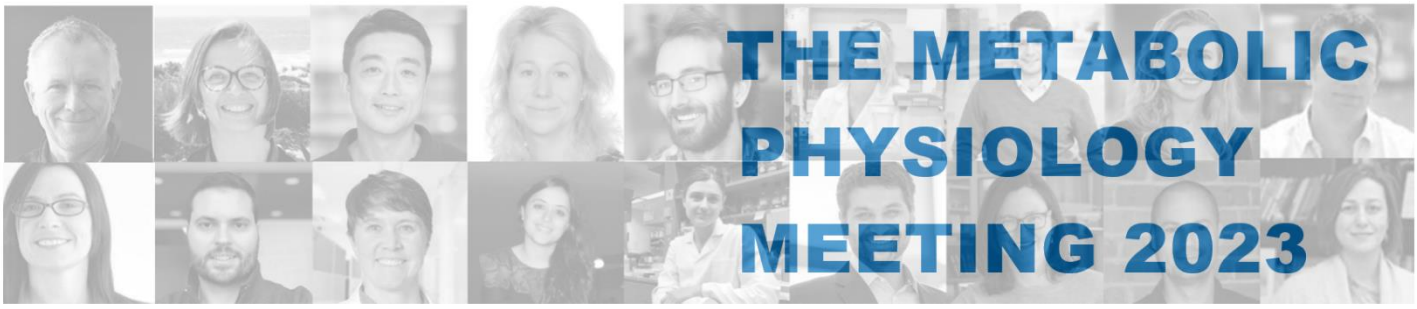

ATOMIC Poster Session Abstracts

POSTER 1.

mTORC1 Controls Postprandial Hepatic Glycogen Synthesis Via Ppp1r3b

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In response to a meal, insulin potently increases hepatic glycogen which contributes to systemic carbohydrate homeostasis. Downstream of insulin, the mechanistic target of rapamycin complex 1 (mTORC1) is an established regulator of hepatic lipid metabolism, autophagy, and protein translation, however its role in hepatic glucose homeostasis is less well characterized. Here, we used comprehensive unbiased metabolomics coupled with mouse genetics to define a new and essential role for liver mTORC1 signaling in the control of postprandial glycolytic metabolites and glycogen deposition. Using a combination genetics and tracing studies, we show that mTORC1 activity is required for glycogen synthase activity and glycogenesis. Mechanistically, hepatic mTORC1 deletion blocked the induction of Ppp1r3b, a well-established type 2 diabetes loci and phosphatase critical for the for activation of glycogen synthase activity and glycogen synthesis. Re-expression of Ppp1r3b in L-Raptor-KO livers partially restored GS activity and postprandial glycogen storage. Collectively, we identify a new role for mTORC1 signaling in the regulation of Ppp1r3b expression and the subsequent control of postprandial hepatic glycogen synthesis.

POSTER 2.

Control of post-prandial metabolism by gut hormone receptor signaling.

Nadya M. Morrow[1,2], Chelsea Klein[1,5], Natasha A. Trzaskalski[1,2], Antonio A. Hanson[1,2], Cassandra A. A. Locatelli[1,2], Serena M. Pulente[1,2], Erin E. Mulvihill[1,2,3,4]

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BACKGROUND: Enteroendocrine cells in the gut epithelium secrete gut hormones in response to a meal to maximize nutrient absorption. These hormones include glucagon-like peptide 1 (GLP-1), GLP-2, and glucose-dependent insulinotrophic polypeptide (GIP). GLP-1 and GIP both amplify glucose-stimulated insulin secretion from pancreatic islets[1] whereas both GLP-1 and GLP-2 promote gut surface area expansion[2, 3]. Controlled delivery of dietary triglycerides from the gut is lost with age and metabolic disease[4, 5]. However, most studies evaluating gut hormone signalling exclusively use male mice. We hypothesize that gut endogenous GLP-1R, GLP-2R, and GIPR signalling integrate the gut response to a meal.

METHODS: Male and female *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$, *Glp1r*$^{-/-}$*Gipr*$^{-/-}$, and wild-type control mice (n=4-6/group/end point) were separated into three experimental gropus: 1) chow diet feeding until 10 weeks of age, 2) chow diet feeding until 30 weeks of age, and 3) high-fat diet feeding until 28 weeks of age. We employed a 24 hour fast-4-hour refeeding protocol to stimulate the release of gut hormones and downstream signalling. We isolated RNA from jejunal tissue after 24 hours of fasting and after 4 hours of refeeding for Nanostring gene expression analyses. Another set of mice were challenged to an oral glucose (2g/kg body weight) and oral mixed meal gavage (200 µL) after a 24 hour fast.

RESULTS: In the gut duodenum, refeeding increased villi length 1.2-fold in male wild-type mice (p= 0.0464) but not in *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ or *Glp1r*$^{-/-}$*Gipr*$^{-/-}$ mice. Duodenal villi length was unchanged in female mice from the fasted to refed state, independent of genotype. Duodenal villi length did not change from fasted to refed state in high-fat diet-fed or aged male or female mice, independent of genotype. Nanostring analyses revealed that refeeding reduced the pathway score of arginine metabolism in wild-type (p= 0.0359) and *Glp1r*$^{-/-}$*Gipr*$^{-/-}$ mice (p= 0.0014) but not in *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice. Male *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ (p= 0.0031 and 0.0280) and *Glp1r*$^{-/-}$*Gipr*$^{-/-}$ mice (p= 0.0078 and 0.0107) displayed a higher pathway score for autophagy and MAPK signalling with fasting compared to wild-type mice. The pathways score for fatty acid synthesis was only reduced with refeeding in *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice (p= 0.0374). In female mice, however, arginine metabolism, autophagy and MAPK signalling pathway scores did not change with refeeding, independent of genotype. Interestingly, the pathway score for fatty acid synthesis was significantly lower with refeeding compared to fasting in wild-type female mice only (p= 0.0073). Oral glucose tolerance tests revealed unchanged glucose tolerance in young male *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice compared to wild-type controls. In females, however, *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice displayed worse glucose tolerance than wild-type controls. Oral mixed meal tolerance tests, by contrast, revealed worse glucose tolerance in both male and female young *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice. By 30 weeks of age, glucose tolerance was worse in *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ mice compared to controls, independent of sex.

CONCLUSIONS: Our data also support the conclusion that GLP-1R signalling enhances gut surface area expansion in male mice, however, this response is lost with age and high-fat diet feeding. Our data suggest that GLP-1R signalling controls autophagy and MAPK signalling in male mice. Additionally, our data suggest that glucose lowering by GIPR signalling is only achieved in young male mice. Understanding the sex-specific contributions of these endogenous peptide hormones to metabolic disease progression will be critical for determining the patient that may benefit from peptide therapy[6].

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POSTER 3.

Mitochondrial Remodeling During Heart Failure Progression in Spontaneously Hypertensive Rats

Scott P Lyons[1]*, Timothy R. Koves[1,2]*, Jonathan Kasper[1], Jordan M. Johnson[1], Dorothy H. Slentz[1], **Paul A. Grimsrud**[1,2], and Deborah M. Muoio[1,2]

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Mitochondrial decline is well recognized as a hallmark of heart failure, which is a leading cause of mortality worldwide. However, the temporal development of mitochondrial dysfunction and its role as a cause or consequence of disease progression remains poorly defined. Here we investigated the relationship between mitochondrial function and age-dependent progression of heart failure using spontaneously hypertensive rats (SHR), which develop hypertension at ~ 2 months of age, whereas heart failure develops in ~50% of animals at ~12-24 months. SHR and age-matched WKY rat controls were studied at 5 months and 18-24 months. Molecular and functional characterization of SHR versus WKY heart mitochondria revealed early changes in mitochondrial quality, including diminished respiratory efficiency and upregulation of the ketolytic enzyme, beta-hydroxybutyrate dehydrogenase (BDH1). Mitochondrial proteomics performed at late-stages of disease followed by PCA and hierarchical clustering identified two distinct SHR subgroups that separated from the WKY controls and predicted differences in cardiac hypertrophy and heart function assessed by echocardiography. Proteins involved in ketolysis, the TCAC, FAO, and BCAA changed uniquely in each of the 3 groups. Proteins and metabolites involved in ketolysis and CoA buffering correlated strongly with fractional shortening. In sum, multi-omics analysis of cardiac mitochondria can be used for prediction of heart failure development status in the SHR model.

POSTER 4

Skeletal muscle mitochondrial efficiency from human subjects with different metabolic state.

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In humans, the metabolic variable that is most affected by extended periods of weight loss or weight gain is non-resting energy expenditure, implying that extended periods of energy deficit or energy surplus induce reciprocal changes in metabolic efficiency in attempt to restore energy balance. The mechanism(s) by which metabolic efficiency is regulated is not known. Mitochondria are the largest source of energy production, but whether mitochondria adjust the efficiency at which energy is produced has historically not been possible to test due to technical limitations. Using a recently developed respiratory clamp technique that recapitulates the interplay among the thermodynamic free energy driving forces governing mitochondrial oxidative phosphorylation (OxPhos) (Fig 1), we tested the hypothesis that mitochondrial bioenergetic efficiency is different between individuals under different chronic metabolic states. Muscle biopsies were obtained after an overnight fast from obese sedentary (n=6) and actively training endurance athletes (n=10). Permeabilized fiber bundles and isolated mitochondria were prepared to assess ex vivo mitochondrial efficiency by creatine kinase force-flow respiratory kinetic and ATP:O ratio analysis using complex I-specific and multi-substrate combinations. Endurance athletes were characterized by significantly higher maximal ADPstimulated rates of mitochondrial oxygen consumption (JO2) and respiratory conductance (slope of JO2 x ΔGATP and -slope of %ΔΨ x ΔGATP) during multi-substrate supported respiration relative to mitochondria from obese sedentary subjects. These data provide the first evidence that skeletal muscle mitochondria from endurance athletes, in addition to greater oxidative capacity, is characterized greater bioenergetic efficiency relative to sedentary obese individuals, implying that efficiency of the mitochondrial OxPhos system is regulated.

POSTER 5.

Exercise Training and Induction of PGC1α Promote Mitochondrial Lipid Tolerance

Jordan M. Johnson[1]*, **Timothy R. Koves**[1,2]*, Ashley S. Williams[1], Scott B. Crown[1], Scott P. Lyons[1], Paul A. Grimsrud[1], and Deborah M. Muoio[1,2]

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Cardiometabolic diseases are strongly associated with an inability to switch between fat and carbohydrate fuel sources termed “metabolic inflexibility”. Metabolic inflexibility is characterized by one or more bottlenecks during fatty acid oxidation (FAO) that promote acylcarnitine accumulation which exacerbates metabolic dysfunction. As mitochondria exhibit diminished FAO capacity, their ability to switch from carbohydrate to fat catabolism deteriorates, promoting metabolic dysfunction at the tissue and whole-body level. In contrast, exercise training improves FAO, promoting greater metabolic flexibility and whole-body metabolic health. Despite the links between metabolic flexibility and cardiometabolic health, the bioenergetic mechanisms remain enigmatic. We sought to understand how skeletal muscle (SkM) mitochondria adapt to promote lipid tolerance in response to PGC1α overexpression or regular exercise. Here we utilize comprehensive bioenergetic, metabolomic and proteomic platforms to explore these mechanisms.

POSTER 6.

Role of the PI3K-mTOR pathway in glucose-stimulated insulin secretion from pancreatic beta cells

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Dysregulation of mechanistic target of rapamycin complex 1 (mTORC1) signaling has been linked to several metabolic diseases, including obesity and type 2 diabetes. mTORC1 senses local (nutrients, stresses) and systemic (growth factors) signals to control cell and tissue growth as well as systemic metabolism. Cell culture studies have found that insulin and growth factors activate mTORC1, in part, via PI3K signaling and the Akt-mediated phosphorylation of TSC2, which is the core functional component of the tuberous sclerosis complex (TSC) protein complex, an essential inhibitor of mTORC1. Akt-dependent phosphorylation of TSC2 inhibits the TSC complex to activate mTORC1. However, the physiological role of this regulation in vivo is unknown.

We have generated a novel conditional mouse model that expresses a TSC2 mutant lacking the 5 known Akt-regulatory sites (TSC2-5A) to specifically disconnect PI3K-Akt signaling from mTORC1 regulation, while retaining normal Akt activation and mTORC1 control by other upstream signals. Surprisingly, mice with whole-body expression of the TSC2-5A allele fully complement the embryonic lethality of *Tsc2*$^{-/-}$ mice, indicating that these phosphorylation sites are dispensable for normal mammalian development. However, relative to littermate TSC2-WT mice, TSC2-5A mice are strongly glucose intolerant, while maintaining insulin sensitivity, suggesting a defect in glucose-stimulated insulin secretion. This metabolic phenotype is associated with reduced mTORC1 activation in primary pancreatic islets isolated from TSC2-5A mice. Previous studies using mouse models with chronic activation or inhibition of mTORC1 in β-cells concluded that its primary role is in controlling β-cell mass and insulin production. However, β-cell mass, islet size and insulin content were unaffected in the TSC2-5A pancreas. Instead, we found that TSC2-5A β-cells are unable to acutely secrete insulin in response to glucose stimulation. Interestingly, TSC2-5A islets can secrete insulin to a level comparable to TSC2-WT when treated with KCl, demonstrating that insulin levels and the secretory machinery are intact. These findings provide novel evidence that the Akt-mediated activation of mTORC1 in pancreatic β-cells is required for proper glucose sensing and metabolism to trigger insulin release. I will present our latest unpublished data on the mechanism underlying this regulation and the phenotype of a β-cell-specific TSC2-5A mice that we have recently generated.

POSTER 7.

Knock-out of NCK1 in pancreatic islets lower basal β-cell burden and enhances β cell response to metabolic stress.

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Preventing pancreatic β-cell failure could help treat diabetes. The unfolded protein response (UPR), is essential for β-cell function. Silencing of *Nck1* in cultured β-cell lines enhances the UPR pathway and improves β-cell function. Therefore, we hypothesized that knockdown of *Nck1* in mouse islets would prevent β-cell failure in diabetes.
We generated mice with a β-cell specific knock-out of *Nck1* (NCK1-βKO mice). Insulin secretion was assessed in KO mice and age/sex-matched littermate Cre controls, fed a chow or high fat/high sucrose (HFHS) diet. UPR pathway was analyzed in primary islets.
Under chow diet, male NCK1-βKO mice had lower insulin secretion in response to a mixed meal challenge compared to control mice. This was consistent with lower glucose-stimulated insulin secretion in isolated primary islets, associated with decreased insulin gene expression and UPR pathway. Female NCK1-βKO mice also had lower insulin secretion in response to a mixed meal and oral glucose, but without changes in the UPR.
Under HFHS diet male NCK1-βKO mice had increased insulin secretion in response to a mixed meal compared to control mice, as well as enhanced induction of insulin gene expression and UPR pathway in primary islets. In contrast female NCK1-βKO mice exhibited similar insulin secretion in response to mixed meal as control mice, despite decreased UPR pathway in primary islets.
Thus, silencing *Nck1* in pancreatic β-cells of healthy mice decreases insulin secretion, which could reduce β-cell burden, whereas it increases insulin secretion during metabolic stress, enhancing β-cell capacity to adapt to diabetogenic stress, but only in males.

POSTER 8.

Weight cycling accelerates pancreatic islet dysfunction in diet-induced obesity.

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Weight cycling (WC) worsens cardiometabolic disease risk. Our group previously found that WC amplifies glucose intolerance compared to equally obese mice that have not weight cycled. This was linked with impaired beta cell function, but the underlying mechanisms are not clear. This study aimed to determine the mechanism(s) by which WC worsens beta cell function. C57BL/6J mice were placed on 9 wk cycles of low fat diet (LFD) or high fat diet (HFD) for a total of 27 weeks to generate lean (LFD x 27 weeks) continuous diet induced obese (C-DIO: LFD x 9 weeks to HFD x 18 weeks) , and WC diet induced obese (WC-DIO: HFD x 9 weeks to LFD x 9 weeks to HFD x 9 weeks). After the 27-week period, pancreatic islets were isolated for perifusions experiments or RNA sequencing. Oral glucose tolerance was worsened in WC-DIO versus C-DIO mice, which we previously found to be accompanied with decreased in vivo insulin secretion. To identify whether weight-cycling causes intrinsic pancreatic dysfunction, islets were isolated and insulin secretion was quantified via perifusion experiments. WC-DIO islets have markedly lower peak insulin secretion than C-DIO islets and reduced insulin content. The dampened insulin response was not related to differences in basal or epinephrine-induced glucagon secretion between WC-DIO and C-DIO islets. RNA sequencing of isolated islets revealed that redox-sensitive thioredoxin-interacting protein (TXNIP) was among the highest differentially expressed genes in WC-DIO. Its elevation has been linked with increased oxidative stress and beta cell dysfunction in rodents and humans with diabetes. Pharmacological suppression of TXNIP using a small molecule inhibitor (SRI-37330) restored glucose tolerance in WC-DIO mice and enhanced insulin secretion. Collectively, these data suggest that TXNIP is a negative regulator of beta cell adaptability to overnutrition in mice.

POSTER 9.

Retro-translation of molecular profiles associated with NASH resistance in humans.

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The molecular underpinnings of non-alcoholic steatohepatitis (NASH) development in patients are poorly understood. To generate new insight into the molecular determinants of NASH, we acquired a set of 60 human liver biopsies from a well characterized bariatric surgery biorepository at the Québec Heart and Lung Institute (QHLI). This sample set represents persons with severe obesity (BMI>40) discordant for NASH, among who 48% had at least one copy (CG/GG) of the PNPLA3 risk allele. Considering their BMI, these I148M carriers represent a population at particularly high risk for NASH. Surprisingly, we identified an atypical group of five such individuals that did not have NASH or severe steatosis. Four out of five of these individuals had T2D or impaired fasting glucose and the mean age was 52.6 years compared to 42.1 in the original set of 60 samples. Hence, the lack of NASH could not be explained by better overall metabolic health or less time for disease progression. Thus, we hypothesized that these individuals express factors in the liver that are protective against NASH. In order to test this idea we identified a well matched population for genotype, BMI, sex, and diabetes status that displayed the expected severe features of NASH, which we termed "CG-NASH Prone" (CG-NP, n=6) to compare with our "CG-NASH Resistant (CG-NR)" group. As part of our search for protective factors we performed small-RNA-Seq which revealed that the microRNA, miR-375, is strikingly enriched in livers from our CG-NASH resistant cohort. We then tested whether adeno-associated virus 8 (AAV8)-mediated miR-375 expression in the liver can reverse the progression of NASH in mice. To induce NASH we fed C57BL/6 mice a choline restricted high fat diet (CR-HFD, 40% fat) supplemented with 30% fructose in the drinking water for five weeks. Hepatic miR-375 expression, was achieved in week five on diet by retro-orbital administration of AAV8-CMV-miR-375 or scrambled control AAV8-CMV-miR375scr. Three weeks following transduction with miR375 or control-miR375-scr AAV8 (n=10/group) livers were harvested, weighed and preserved for histologic and biochemical analyses. Liver from mice receiving AAV8-CMV-miR375 weighed significantly less, had smaller lipid droplets, and 50% lower expression of the inflammatory cytokine TNFalpha. Two other predicted miR375 targets PTSD22 and RASD also showed lower expression levels. These data suggest that higher expression of miR-375 in liver is protective against NASH.

POSTER 10.

Discovery of novel multi-domain enzymes that function in atypical lipid catabolism.

Eddie Rashan.

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Fatty acid β-oxidation (FAO) drives the catabolism of fatty acids to yield energetic intermediates and buffer lipotoxic stress under various physiological demands (e.g., when glucose becomes limiting). Cells rely on a network of enzymes to efficiently degrade structurally and chemically diverse fatty acids, however our understanding of the enzymatic machinery and mechanisms that underlie FAO remains incomplete. Here, we present our discovery and characterization of poorly studied enzymes that participate in the catabolism of atypical, oxidized lipid species in mammals. Leveraging in vitro biochemistry and cellular metabolomics, we determine that these enzymes act bifunctionally to catalyze the entry and catabolism of a representative substrate through the canonical FAO pathway via a novel mechanism. Despite having similar enzymatic activities in vitro, we provide evidence that these enzymes do not function redundantly in the catabolism of distinct species of this atypical lipid class in both cell culture and mouse models. Overall, this work advances our understanding of how atypical fatty acid metabolism is regulated and how dysfunction of these poorly understood enzymes may underlie human health and disease.

POSTER 11.

Role of the Skeletal Muscle Androgen Receptor in Regulating Metabolism in Male Mice

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BACKGROUND: Obesity is associated with impaired glucose metabolism and insulin resistance which are thought to play a role in a variety of obesity-linked comorbidities. These metabolic impairments are evident in the skeletal muscle as it is the major source of insulin-stimulated glucose uptake. Thus, developing muscle-centric therapies is a viable option to remedy the metabolic perturbations seen in obesity. Administration of exogenous sex steroids has previously been shown to beneficially impact metabolic processes, however, the off-target effects and potential negative health risks associated with global steroid therapies hinder their use clinically. Therefore, there is a critical need to understand the tissue-specific effects of steroid action to develop targeted therapeutics which bypass any potential unwanted side effects while also delivering efficacious outcomes.

PURPOSE: The purpose of this experiment was to interrogate the role of the skeletal muscle androgen receptor (AR) in regulating metabolic outcomes and the therapeutic potential of AR manipulation to mitigate impaired glucose metabolism in the setting of diet-induced obesity.

METHODS: We developed a novel mouse model that allow for inducible deletion of skeletal muscle AR (SkM-AR^{ID}). Male mice were fed either a purified LFD or HFD for 19 weeks. Body weight was assessed throughout the course of the experiment, while body composition (DEXA), glucose tolerance tests, and insulin tolerance tests were performed near the termination of dietary treatment.

RESULTS: Skeletal muscle AR inducible deletion improved glucose tolerance independent of changes to adiposity.

CONCLUSION: Modulation of both skeletal muscle AR may serve as viable therapeutic option for the treatment of obesity-linked metabolic impairments in males. More research is needed to fully understand the molecular mechanisms responsible for these efficacious therapeutic outcomes.

POSTER 12.

Impaired protein synthesis due to decreased hypusination of EIF5A led to dysfunctional mitochondria in NAFLD

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Impaired autophagy and dysfunctional mitochondria are known molecular mechanism(s) underlying impaired lipid utilization in various metabolic disorders including non-alcoholic steatohepatitis (NASH). Spermidine is a natural polyamine that has health benefits and extends life span through induction of autophagy. It also is the sole substrate for the post-translational hypusination of the translation factor EIF5A (EIF5A^{H}). Although hypusination of EIF5A is conserved in all eukaryotic cells, little is known about its dysfunction in human diseases. Here, we have found that hepatic mRNA level of *DOHH,* the last enzyme that utilize spermidine to catalyze the hypusination of EIF5A, is decreased in patients and mice NASH, and hepatic cells treated with fatty acids. The mouse and cell culture models of NASH have concomitant decreases in Eif5a^{H}, autophagic and mitochondrial protein synthesis which leads to lower mitochondrial activity and fatty acid β-oxidation. Spermidine treatment restores EIF5A^{H}, partially restores protein synthesis, autophagy, and mitochondrial function in NASH, and prevents NASH progression *in vivo*. Thus, the disrupted DHPS-DOHH-EIF5A^{H} pathway during NASH represents a therapeutic target to restore hepatic protein synthesis and mitochondrial fatty acid oxidation (FAO), and prevent NASH progression.

POSTER 13.

The Role of Manganese in Liver Lipid Metabolism and NAFLD

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The liver is a hub for metabolic processes, regulating cholesterol, lipid and lipoprotein homeostasis in addition to producing bile that clears organic and inorganic compounds out of the body. The role for inorganic compounds, particularly metals, within the mammalian system is profoundly understudied despite our daily ingestion and exposure to them. Manganese (Mn) is an essential trace element and is exclusively acquired through the diet. The canonical role of Mn is to act as an enzyme cofactor, required for numerous physiological processes. For example, phosphoenolpyruvate carboxykinase (PEPCK) is a Mn-containing regulator of gluconeogenesis (Lee 1981) and arginase, which catalyzes the final stage of the urea cycle, requires Mn for activity (Ash 2004). However, accumulation of excess Mn in the body can lead to toxicity. Rare familial genetic variants in the Mn efflux transporter (SLC30A10) results in hypermanganesemia and chronic liver diseases, including steatosis and cirrhosis (Tuschl 2012, Quadri 2012). Given this observation, we aimed to discern the impacts Mn accumulation has on hepatic lipid metabolism. We used mice with knockout of Slc30a10 in the intestinal epithelium and hepatocytes as a model of liver Mn accumulation. After keeping these mice on a low-fat chow diet, we found that mice with Mn accumulation have a significant increase in hepatic triglyceride content and modest increases in fibrosis-associated gene expression. These data demonstrate that Mn status is positively associated with steatosis leading us to ask the reverse question: do chronic liver diseases, such as non-alcoholic fatty liver disease (NAFLD), affect Mn homeostasis? We fed mice a high-fat, choline-deficient L-amino acid defined diet (CDAA) to induced NAFLD pathologies and assessed changes in hepatic gene expression of Mn transporters and metal content. Strikingly, we found that *Slc30a10* was downregulated >90% in CDAA-fed mice compared to control mice. Consistent with this, we found a significant increase in both the concentration and total amount of Mn in liver tissue of CDAA-fed mice compared to Mn-matched control diet-fed mice. We found that the decrease in *Slc30a10* expression can be explained in-part due to fibrosis, but not inflammation, although the complete mechanism remains unknown at this time. These data shed light on a novel role for Mn in regulating hepatic lipid metabolism, and how mouse models of NAFLD are associated with changes in hepatic Mn content and *Slc30a10* expression.

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POSTER 14.

Impact of Hepatic ER-alpha Overexpression on development of Obesity in Female Mice

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INTRODUCTION: Women experience greater protection from obesity-related comorbidities including insulin resistance and hepatic steatosis compared to males. Evidence suggests that the primary pre-menopausal estrogen, 17β-estradiol (E2), serves as the regulator of this protection. The exact mechanism by which E2 controls protection of obesity-related comorbidities is unknown Therefore, it is critical to identify the tissues and pathways responsible for eliciting this protective effect.

METHODS: Our lab has developed a novel Tet-On estrogen receptor-α (ERα) liver-specific mouse model (LIV-ERα$^{\uparrow}$). This mouse model allows for the inducible overexpression of liver ERα. We used this model to determine if overexpression of hepatic ERα could prevent the development of obesity and associated metabolic dysfunction in female mice. Female wildtype littermate control and LIV-ERα$^{\uparrow}$ mice were fed either a purified low-fat or high-fat diet (HFD) for 20 weeks (n=15-19/group). Body composition and metabolic outcomes were assessed.

RESULTS: Hepatic ERα overexpression improved glucose metabolism independent of changes to body composition or adiposity.

CONCLUSIONS: Targeting hepatic ERα may be a potential therapeutic target for the treatment of obesity-associated metabolic dysregulation. Future studies should be undertaken to determine the molecular mechanisms by which hepatic ERα signaling improves metabolic outcomes.

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POSTER 15.

Application of the creatine kinase energetic clamp technique to liver mitochondria highlights the interplay between hepatic ketogenesis and respiratory performance.

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During periods of prolonged fasting and/or increased lipid supply, hepatic mitochondria engage their uniquely robust capacity to direct carbon fuels and redox potential towards ketone production and efflux. The overarching goal of this project was to develop a workflow that permits comprehensive assessment of bioenergetics in liver mitochondria exposed to physiologically relevant substrate supply and energy demands. To this end, we adapted our previously established respiratory diagnostics platform featuring the creatine kinase (CK) clamp technique to investigate the interplay between fatty acid-supported bioenergetics and mitochondrial ketogenic capacity. After optimizing assays to assess mitochondrial ketone production in parallel with respiratory fluxes, we proceeded to examine the impact of an 18 h overnight fast on both endpoints. As anticipated, fasting increased fatty acid-supported oxygen flux (JO2), which was accompanied by enhanced mitochondrial ketogenesis. Interestingly, subsequent analysis of the mitochondrial proteome via label-free proteomics revealed that the fasting-induced mitochondrial phenotype was not accompanied by increased abundance of fatty acid oxidation (FAO) or ketogenic proteins, implying possible regulation by posttranslational protein modifications. Whereas FAO is known to play an essential role in supplying the acetyl CoA substrate needed for ketone production, we questioned whether flux through the first and/or last steps of ketogenesis, catalyzed by hydroxymethylglutarylCoA synthase 2 (HMGCS2) and beta-hydroxybutyrate dehydrogenase 1 (BDH1), respectively, might have a direct impact FAO-supported bioenergetics. We therefore assessed energy transduction and ketogenesis using freshly isolated liver mitochondria from 18 h fasted mice with hepatocyte-specific deletion of BDH1 (BDH1Alb) as compared with littermate controls (BDH1fl/fl). As anticipated, production of beta-hydroxybutyrate (but not acetoacetate) was decreased in mitochondria from BDH1Alb mice. Deletion of BDH1 resulted in a modest reduction in octanoyl-carnitine-supported JO2, but had little effect on palmitoyl-carnitine supported energetics, including mitochondrial redox potential. By contrast, when we blocked the first committed step in ketogenesis using the HMGCS2 inhibitor, hymeglusin (HG), the resulting bioenergetic phenotype was quite striking. Thus, HG strongly inhibited fatty acid-supported JO2 while increasing mitochondrial redox potential, regardless of chain length. To investigate the mechanisms contributing to these observations, we combined the CK clamp technique fixed at an intermediate energy demand with MS-based metabolomics. The analysis showed that inhibition of HMGCS2 leads to robust accumulation of several even chain acyl CoA intermediates, profound depletion of free CoA, and corresponding disruptions in TCAC flux. In sum, we developed a workflow for exploring the interplay between ketogenesis and respiratory fluxes in liver mitochondria. The findings show that overnight fasting enhances the capacity of liver mitochondria to perform FAO-supported respiration and ketogenesis without overt upregulation of these pathways at the level of the mitochondrial proteome. Moreover, we provide strong evidence that flux through the ketogenic pathway plays a prominent role in recycling of the mitochondrial free CoA pool, such that disruption of a key proximal step in ketogenesis leads to CoA trapping in FAO intermediates, a catastrophic decline in free CoA, and profound perturbations in fatty acid-supported bioenergetics. These findings set the stage for future studies aimed at understanding the role ketogenic failure in the context of metabolic diseases associated with impaired hepatic FAO flux.

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POSTER 16.

Optimizing pancreatic pseudoislet generation for cell-specific viral transduction

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Background and aims: Impairments in human pancreatic islet function play a major role towards the development of diabetes. As such, significant research has been conducted to better understand human islet biology. However, due to the multicellularity and the general impermeability of pancreatic islets to experimental reagents, studies involving genetics and intercellular crosstalk has been challenging1. Thus, the dispersion of islets into single cell populations and their subsequent reaggregation into "pseudoislet" organoids has been a powerful experimental tool that has allowed researchers to genetically manipulate human islets. Although this technique is widely used, there are currently no experimental techniques developed to separate different live islet cell populations to either manipulate pseudoislet composition or perform cell-specific genetic modifications. Therefore, our aim is to first optimize a protocol to generate pseudoislets that resemble native islets and then introduce magnetic-activated cell sorting (MACS) as a method to isolate pancreatic alpha cells for cell-specific viral transduction. Methods: Primary mouse islets were isolated from 10-week-old male C57BL/6J mice as previously described2. Human pancreatic islets were obtained from the Alberta Diabetes Institute IsletCore. To generate pseudoislets, ~500 mouse or human islets were handpicked and digested with 0.25% Trypsin. Dispersed islet cells were resuspended in RPMI-1640 (11.1 mM glucose, 10% FBS, 1% penicillin/streptomycin, 2 mM HEPES, 1 mM sodium pyruvate) supplemented with VascuLife endothelial mix, then seeded in ultra-low adherence 96-well plates for 4 days. Alternatively, dispersed islets were incubated with anti-CD26 antibody bound to anti-mouse IgG magnetic beads, following which a magnetic column was used to separate out alpha cells and immunohistochemical analysis was conducted. Islet perifusions were performed as previously described3. Results: Our protocol generated both mouse and human pseudoislets that have similar diameters as their native counterparts. In addition, the pseudoislets were functionally comparable to native islets as indicated by similar glucose-stimulated and glucagon-like peptide 1-mediated insulin secretion. Amino acid and glucose-dependent insulintropic polypeptide also stimulated similar glucagon secretion in pseudoislets compared to native islets. Interestingly, despite their functional similarities, pseudoislets had lower insulin content versus native islets. Using MACS, we successfully isolated pancreatic alpha cells, which was validated using immunohistochemical staining of insulin and glucagon. Conclusions: Our findings demonstrate that our pseudoislets mimicked native islets in terms of their morphology and dynamic responsiveness to glucose and other stimuli. We also illustrated that MACS could be used as a potential method to purify live cell populations and allow for cell-specific gene manipulation, as well as alterations of pseudoislet cell composition.

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POSTER 17.

Thermoneutral housing produces greater increases in energy expenditure during wheel running in mice

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Background: Voluntary wheel running (VWR) is used to increase mouse physical activity, and hypothetically energy expenditure (EE). However, this may be confounded by the observed ~2-fold increase in mouse resting EE at standard, sub-thermoneutral housing temperatures. Herein, we used 20ºC & 30ºC housing temperatures to investigate the impact of divergent baseline EE on the capacity of VWR to change EE and mediate metabolic phenotypes. **Methods:** We performed indirect calorimetry experiments in male C57Bl/6J mice housed at 20ºC or 30ºC with VWR or without (SED) to assess total EE and the components of EE during 7-day low-fat (LFD) and high-fat, high-sucrose (HFHS) diets. **Results:** As expected, LFD mice housed at 30ºC have ~40% lower total EE and energy intake, and 60% lower resting EE compared to 20ºC mice. Interestingly, activity EE was ~30% greater in 30ºC mice for both VWR & SED. Importantly, while total activity was increased in VWR, no difference in total activity was observed due to temperature within SED or VWR. VWR increased total EE compared to SED regardless of temperature. VWR did not alter resting EE, which represented ~70% and ~50% of total EE in 20ºC & 30ºC mice, respectively. VWR increased activity EE ~55% at both temperatures. However, activity EE represented greater than twice the percent of total EE in 30ºC mice. During subsequent HFHS feeding, 7-day weight gain was reduced ~40% in male VWR mice regardless of temperature, with VWR having no impact on female diet-induced weight gain. **Conclusions:** While VWR reduced HFHS-induced weight gain at both temperatures, 30ºC mice had a greater VWR-mediated increase in total EE, absolute activity EE and as a percent of total EE. These data suggest that thermoneutral housing is more appropriate for studying the impact of VWR, and increases in EE, on metabolic disease outcomes.

POSTER 18.

Maternal exercise during lactation promotes lipogenic metabolism, sparing glucose for milk.

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Introduction: Breastmilk benefits infants and lactation benefits mothers, but women with obesity often have difficulty initiating and maintaining lactation leading to poor milk production. Exercise improves maternal metabolic conditions such as gestational diabetes, excessive gestational weight gain, and pre-eclampsia during pregnancy, but little is known about its benefit during lactation. In humans, we found maternal exercise induced genes consistent with Prolactin Receptor activation and lipogenesis, suggesting exercise during lactation might promote healthy milk production.

Method: We tested the hypothesis that moderate bouts of maternal exercise would improve metabolic and lactational phenotypes. Pregnant mice were randomized into cohorts of exercise (EX) vs. non-exercise (NEX). On lactation day two (Lac2), litters were standardized to 7 pups per dam. On Lac7, body weights and body composition of all dams were quantified by qMR, as were their independent litters. On Lac7-12, dyads began indirect calorimetry (IDC) to measure whole animal metabolism, energy expenditure, energy balance, respiratory exchange ratio, energy intake, and water consumption. On Lac8, the EX. cohort began a 30min/day moderate exercise bout (12m/min by treadmill) for 4 days. On Lac12, endpoint qMR was performed.

Results: Exercise significantly increased maternal energy balance ($p<0.0001$), total energy intake ($p=0.0142$), and the respiratory exchange ratio (RER) ($p=0.0484$). Overall differences in RER occurred during the light cycle ($p=0.0054$) when dams were inactive, and RER reached >1 indicating lipogenesis. EX cohort spared glucose measured during 13C6-glucose tracer administration, with significantly lower 13CO2 emission following injection of the 13C6 glucose (2mg/kg). Medium chain fatty acids synthesized by the mammary gland were significantly increased in the EX group ($p=0.03$). No differences in maternal or offspring body weight, lean and fat mass, and blood glucose levels between EX and NEX groups were observed.

Conclusion: Our findings indicate that moderate maternal exercise during lactation could enhance de novo fatty acid synthesis for milk by conserving maternal glucose fuel utilization.

POSTER 19.

A hexokinase force-flow technique measuring ATP/O ratios across demand states.

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Mitochondrial ATP/O ratios (P/O) are a measure of the efficiency of both proton conduction and ATP synthesis of the mitochondria making it a useful measurement to study mitochondrial respiration efficiency. The mitochondria electron transport system (ETS) is governed by the interplay between three free energy driving forces: 1) the difference in reduction potential energy between the O2/H2O and NAD+ /NADH redox couples (ΔGredox) which sets the pull on electron flux through the ETS, 2) the proton motive force (ΔGpmf) generated across the mitochondrial inner membrane as electrons flow through the ETS driven by ΔGredox, and 3) the phosphorylation potential (ΔGATP) generated by ΔGpmf driven by proton conductance through ATP synthase. ΔGpmf exerts backpressure on the proton pumps to regulate electron flux through the ETS, and ΔGATP exerts backpressure on ATP synthase and ΔGpmf. Mitochondrial ATP/O ratio (P/O) is a measure of the rate of ATP production (JATP) relative to the rate of O2 utilization (JO2), and has traditionally been used as an index of oxidative phosphorylation (OxPhos) efficiency. We previously developed a hexokinase enzyme-linked system capable of simultaneously measuring both JATP and JO2 across varying ATP concentrations. However, this approach does not generate a ΔGATP and thus does not sufficiently recapitulate the system under which OxPhos efficiency is regulated. The goal of this study is to create a technique to simultaneously measure the mitochondrial P/O values while accounting for the regulating force of ΔGATP. We initially employed a creatine kinase (CK) clamp system and force-flow analysis to measure JO2 across varying demand states at clamped ΔGATP values that more closely model in vivo conditions. Preliminary data provides evidence that the efficiency of JO2 changes across ΔGATP. However, it is not possible to simultaneously monitor JATP with the CK clamp, limiting the ability to directly assess P/O at each ΔGATP (i.e., clamped rate of energy demand). To overcome this limitation, we returned to the hexokinase clamp system, se\ng the initial ΔGATP to the lowest demand state (i.e., high negative ΔGATP), and then step-wise increasing OxPhos demand by titrating in HKII. All components of the assay have been systematically tested and standardized, and preliminary experiments confirm the utility of this approach. Development of a technique to measure P/O that faithfully recapitulates the interplay between driving forces allows, for the first time, to determine whether mitochondrial bioenergetic efficiency during increasing metabolic demand is regulated by different physiological states and/or disease conditions.

POSTER 20.

It Takes Two to Tango: Modulation of Cardiovascular Function with Combination DPP4 Inhibitor and Metformin Treatment

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Currently, 11.7 million Canadians live with diabetes or prediabetes where cardiovascular disease, including ischemic heart disease, remains a major cause of death. One drug therapy used to treat patients are dipeptidyl peptidase-4 (DPP4) inhibitors, which inhibit its enzymatic action to prevent degradation of the incretin hormones to regulate glycemia. Meta-regression analysis of DPP4 inhibitor cardiovascular endpoint trials demonstrated that combination therapy with metformin was cardioprotective compared with inhibitor monotherapy. High circulating levels of DPP4 are associated with severe heart failure and death and interestingly, DPP4 protein levels increase in circulation upon inhibitor treatment. Preclinical studies suggest metformin inhibits DPP4 activity or reduces shedding from organ depots into circulation. Therefore, we hypothesized that combination therapy could modulate cardiovascular risk by reducing the increase in circulating DPP4 which occurs with inhibition.

Wildtype (*Dpp4*+/+), whole-body knockout (*Dpp4*-/-), hepatocyte-specific knockout (*Dpp4hep*-/-) or control (*Dpp4GFP*), and endothelial cell-specific knockout (*Dpp4EC*-/-) and control (*Dpp4EC*+/+) mice were aged, fed a high-fat high-cholesterol diet, and treated with sitagliptin (DPP4 inhibitor), metformin or a combination. Mice were subjected to coronary artery ligation surgery to induce permanent ischemic injury. Prior to, and after surgery, we performed echocardiography to assess cardiac function and akinetic length and collected blood to assess DPP4 activity and soluble protein levels (sDPP4). Our *Dpp4*-/- mouse model was validated by undetectable DPP4 activity and sDPP4, while activity decreased and sDPP4 increased with sitagliptin treatment in *Dpp4*+/+ mice. Similar trends were detected in *Dpp4GFP* mice, with a significant (70%) decrease in activity in *Dpp4hep*-/- mice, revealing the liver as the main source of active DPP4. The increase in sDPP4 with sitagliptin treatment was modestly attenuated, suggesting EC-DPP4 as the main contributor to sDPP4 levels. This phenomenon was confirmed in *Dpp4EC*-/- mice where sDPP4 levels were significantly decreased, but activity was only decreased by sitagliptin and combination treatment in *Dpp4EC*+/+ mice. Lastly, metformin action was confirmed in *Dpp4*+/+ mice, where combination treatment increased AMPK phosphorylation.

Four weeks after surgery, there were no improvements in ejection fraction, except in *Dpp4hep*-/- vs *Dpp4GFP* mice with metformin. Further, left ventricular area and volume were significantly decreased *Dpp4hep*-/- mice vs *Dpp4GFP* mice, and area was significantly decreased in *Dpp4EC*-/- mice vs *Dpp4EC*+/+ mice treated with combination therapy, suggesting prevention of ventricle dilation. Myocardial kinesis was conserved in *Dpp4*-/- vs *Dpp4*+/+ mice, as previously reported. Metformin treatment benefitted *Dpp4hep*-/- mice, however, combination treatment greatly benefitted *Dpp4EC*-/- mice with most possessing a highly kinetic myocardium (85% vs 66% *Dpp4EC*+/+).

In conclusion, cardiac architecture and ventricular kinesis improved in *Dpp4EC*-/- mice with combination therapy. These data suggest that metformin and DPP4 inhibitors are cardioprotective in the absence of EC-DPP4 and may work by targeting hepatocyte derived DPP4.

POSTER 21.

Pax7-Cre deletion of Cox6a2 results in altered mitochondrial function and attenuates ischemic muscle survival and regeneration

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ECU

Chronic limb threatening ischemia (CLTI) is the most severe clinical manifestation of peripheral artery disease (PAD), and is associated with severe limb skeletal muscle myopathy, gangrenous tissue necrosis, and high mortality rates. CLTI is the leading cause of major amputations in the US and there is a need to develop novel therapies. Mitochondria are a key descriptor of CLTI myopathy, although we understand very little about their role in determining disease severity. We developed a lifelong knockout model of Cox6a2, a protein binding subunit in Cytochrome C Oxidase (complex IV of the mitochondrial electron transport system) and a genetic determinant of tissue loss in preclinical models of PAD. We hypothesized that skeletal muscle Cox6a2 is necessary for tissue regeneration after hindlimb ischemia (HLI). Muscle contractile ability, mitochondrial enzyme activity, and skeletal muscle morphology were assessed at baseline and after HLI. Cox6a2 deletion using a Pax7-Cre promoter at baseline reduced oxygen consumption rates of complexes I and II in skeletal muscle mitochondria. After 7-days of HLI, genetic deletion of Cox6a2 resulted in further reductions in mitochondrial function and muscle force production. Extended (28-day) HLI revealed Cox6a2 deletion to be detrimental to limb muscle morphology indicative delays in regeneration from the initial ischemic insult post-surgery. Together, these data indicate that Cox6a2 is required for efficient limb muscle mitochondrial respiration, enzyme activity, and muscle regeneration during acute and prolonged ischemic events.

POSTER 22.

Characterization of Inducible Mature Skeletal Muscle Cox6a2 Loss

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Peripheral artery disease (PAD) is caused by an atherosclerotic lesion that restricts blood flow to the lower extremity. Chronic limb threatening ischemia (CLTI) is the most severe manifestation of PAD, and is associated with gangrenous lesions, necrosis, high rates of limb loss, reduced quality of life, and mortality. Despite the significant presence of PAD, there have been no advancements in treatment options in the last two decades. The treatment options that do exist have been largely unsuccessful at improving limb outcomes, particularly for patients with CLTI. This suggests that blood flow alone is not sufficient for restoration of muscle functional ability. Mitochondrial dysfunction is a common characteristic of patients with CLTI, though little is understood about their contribution to the ischemic limb environment. Previous work in our lab has shown attenuated expression of cytochrome c oxidase subunit 6a2 (Cox6a2), a structural protein within complex IV of the mitochondria in patients with CLTI. We hypothesize that Cox6a2 is necessary for regeneration of mature skeletal muscle in the ischemic environment. To test this hypothesis, we first evaluated different strains of mice with varying susceptibility to ischemic injury following a preclinical model of PAD for paw perfusion recovery, oxygen consumption rates (OCR), and Cox6a2 protein content. BALB/c mice exhibited reduced paw perfusion and Cox6a2 expression. From these data, we generated an inducible murine model of Cox6a2 loss in mature skeletal muscle (HSAMCM; Cox6a2f/f) on a BL6/J background. Post-tamoxifen injection these mice were evaluated at baseline and following a pre-clinical model of PAD for skeletal muscle contractile ability, blood flow restoration, muscle morphology, and mitochondrial function. The Cox6a2 knockout (KO) mice displayed a bioenergetic phenotype and had significantly reduced oxidative respiratory capacity across all substrate conditions at baseline when compared with the wildtype (WT) animals despite having no differences in contractile function. Morphologically, Cox6a2 KO animals had larger cross-sectional area of the tibialis anterior muscle than the WT animals with no differences in capillary density or changes in fiber type. Post-ischemic injury, significant differences in the infarct area and in force production were observed in the KO animals compared with the WT, despite no differences in paw perfusion between the groups.

POSTER 23.

Reactive nitrogen species inhibit branched chain alpha-ketoacid dehydrogenase complex and impact muscle cell metabolism.

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Branched chain α-ketoacid dehydrogenase complex (BCKDC) is the rate limiting enzyme in branched chain amino acid (BCAA) catabolism, a metabolic pathway with great importance for human health. BCKDC belongs to the mitochondrial α-ketoacid dehydrogenase complex family, which also includes pyruvate dehydrogenase complex (PDHC) and oxoglutarate dehydrogenase complex (OGDC). Here we revealed that BCKDC can be substantially inhibited by reactive nitrogen species (RNS) via a mechanism similar to what we recently discovered with PDHC and OGDC — RNS cause inactivating covalent modifications of the lipoic arm on its E2 subunit. In addition, we showed that such modifications on the E2 subunit of α-ketoacid dehydrogenase complexes can further promote inhibition of their E3 subunits. We examined the impacts of this RNS-mediated BCKDC inhibition in muscle cells, an important site of BCAA metabolism, and demonstrated that the nitric oxide production induced by cytokine stimulation leads to a strong inhibition of BCKDC activity and BCAA oxidation in myotubes and myoblasts. More broadly, nitric oxide production reduced the level of functional lipoic arms across the multiple α-ketoacid dehydrogenases and led to intracellular accumulation of their substrates (α-ketoacids), reduction of their products (acyl-CoAs), and a lower cellular energy status. This work revealed a new mechanism for BCKDC regulation, demonstrated its biological significance, and elucidated the mechanistic connection between RNS-driven inhibitory modifications on the E2 and E3 subunits of α-ketoacid dehydrogenases. Together with previous work, we revealed a general mechanism for RNS to inhibit all α-ketoacid dehydrogenases, which has numerous physiological implications across multiple cell types.

POSTER 24.

Cardiovascular Impact of Global BCAA Catabolic Regulation

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Pharmacological activation of branched-chain amino acid (BCAA) catabolism showed a potent effect against heart failure. However, the specific protective mechanisms of BCAA catabolism activation are still ambiguous. As part of the long-term efforts to establish mechanism of action for this therapeutic strategy, we propose to establish global BCKDK knockout mice. However, our results showed that global BCAA activation by genetic BCKDK knockout doesn't prevent cardiac dysfunction post pressure overload, the effect is different with pharmacological activation. Surprisingly, our results found that global BCAA activation improves survival. To further explore the protective mechanism of pharmacological activation of BCAA catabolism, we used BCKDK inhibitor BT2 to activate BCAA catabolism, Telemetry data showed that pharmacological activation of BCAA catabolism lowers systolic blood pressure, diastolic blood pressure and mean arterial pressure, perhaps explaining the observed cardioprotection.

POSTER 25.

Mitochondrial Pyruvate Carrier Inhibition Initiates Metabolic Crosstalk to Stimulate Branched Chain Amino Acid Catabolism

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The mitochondrial pyruvate carrier (MPC) has emerged as a therapeutic target for treating insulin resistance, type 2 diabetes, and nonalcoholic steatohepatitis (NASH). We evaluated whether MPC inhibitors (MPCi) might correct impairments in branched chain amino acid (BCAA) catabolism, which are predictive of developing diabetes and NASH. In a recent randomized, placebo-controlled Phase IIB clinical trial to test the efficacy and safety of the MPCi MSDC-0602K (EMMINENCE; NCT02784444) circulating BCAA concentrations were measured in people with NASH and type 2 diabetes. In this 52-week trial, patients were randomly assigned to placebo (n = 94) or 250 mg MSDC-0602K (n = 101). In people treated with MSDC-0602K, which led to marked improvements in insulin sensitivity and diabetes, plasma concentrations of BCAAs were significantly decreased compared to baseline, while placebo had no effect. The rate-limiting enzyme in BCAA catabolism is the mitochondrial branched chain ketoacid dehydrogenase (BCKDH), which is deactivated by phosphorylation.

To test the direct effects of various MPCi on BCAA catabolism in vitro, we used multiple human hepatoma cell lines and mouse primary hepatocytes. We found that MPCi markedly reduced BCKDH phosphorylation and stimulated branched chain keto acid catabolism; an effect that required the BCKDH phosphatase PPM1K. Mechanistically, the effects of MPCi were linked to activation of the energy sensing AMP-dependent protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) kinase signaling cascades in vitro. Lastly, we investigated how hepatocyte-specific deletion of MPC2 affects BCAA metabolism in the liver of obese mice and MSDC-0602K treatment of Zucker diabetic fatty (ZDF) rats. BCKDH phosphorylation was reduced in liver of obese, hepatocyte-specific MPC2 knockout (LS-Mpc2-/-) mice compared to wild-type controls concomitant with activation of mTOR signaling in vivo. Finally, while MSDC-0602K treatment improved glucose homeostasis and increased the concentrations of some BCAA metabolites in ZDF rats, it did not lower plasma BCAA concentrations. Collectively, these data demonstrate novel cross talk between mitochondrial pyruvate and BCAA metabolism and suggest that MPC inhibition leads to lower plasma BCAA concentrations and BCKDH phosphorylation by activating the mTOR axis. However, the effects of MPCi on glucose homeostasis may be separable from its effects on BCAA concentrations.

POSTER 26.

Loss of CD47 alters CD8+ T cell activation in vitro and immunodynamics in mice.

Dipasmita Pal Nath

NIH

CD47 is a transmembrane glycoprotein ubiquitously expressed in different organs and tissues that regulates cellular responses to stress. CD47 has established roles in the immune system for regulating macrophage phagocytosis and lymphocyte activation, with growing evidence of its cell-intrinsic regulatory roles in T cells and natural killer cells. Using in vitro and in vivo models, we show here that CD47 differentially regulates CD8+ T cell responses to short- versus long-term activation. Following early activation in vitro, short-term stimuli elevated pathogen-reactive gene expression and enhanced proliferation and the effector phenotypes of CD47-deficient relative to CD47-sufficient CD8+ T cells. In contrast, persistent TCR stimulation limited the effector phenotypes of cd47−/− CD8+ T cells and enhanced their apoptosis signature. CD8+ T cell expansion and activation in vivo induced by acute lymphocytic choriomeningitis virus (LCMV) infection did not differ in the absence of CD47. However, the frequency and effector phenotypes of cd47−/− CD8+ T cells were constrained in chronic LCMV-infected as well as in mice bearing B16 melanoma tumors. Therefore, CD47 regulates CD8+ T cell activation, proliferation, and fitness in a context-dependent manner. Previously, we have shown CD47-dependent alterations in mitochondrial metabolites including basal citrate and altered citrate synthase levels were identified in WT versus CD47-deficient Jurkat T cells in the absence and presence of stress induced by ionizing radiation. Similar to T cells, we have also found the absence of CD47 in NK cells in the murine model alters mitochondrial metabolism in NK cells. Loss of CD47 results in a defect in mitochondrial metabolism, proton leak, ROS, and increased apoptosis of activated mouse NK cells. Furthermore, a global metabolomics study on the IL15-treated activated NK92 cells indicated nucleotide and lipid metabolism changes after blocking the CD47 with B6H12. Thus, CD47 plays an important role in the activation, metabolic fitness, and immune response in T cells as well as NK cells studied under various platforms.

POSTER 27.

IL-17 influences carbohydrate utilization and lipid metabolism in a sex-dependent manner.

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The interleukin-17 (IL-17) cytokine family has an established role in orchestrating barrier defense, neutrophil recruitment, and inflammatory disease. Recently, the two principal members of this family, IL-17A and IL-17F, have been shown to regulate cellular and systemic metabolism. The molecular mechanisms underpinning this immunometabolic axis are not well defined, however. Here, we used genetic, dietary, and pharmacological methods to elucidate the metabolic actions of IL-17A and IL-17F in liver and adipose tissue. IL-17A had a significant impact on carbohydrate utilization and fatty acid synthesis, effects which were most prominent during the postprandial state and following sucrose ingestion. Unexpectedly, we found that biological sex was a critical determinant of IL-17's impact on thermoregulation, substrate utilization, and lipid synthesis in brown adipose tissue and liver. At the whole-body level, *Il17af* -/- male mice resisted sucrose-induced weight gain. By contrast, IL-17A/F were dispensable for this form of weight gain in female mice. Our findings highlight IL-17 as an important player in directing metabolic and immune homeostasis and raise questions regarding sex differences in these pathways.

POSTER 28.

Targeting mitochondrial membrane potential with organic cations enhances the anti-leukemic effects of BLC-2 inhibition

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Despite initial therapy-induced remission, over 70% of patients suffering from acute myeloid leukemia (AML) will relapse. Relapse in AML is caused by the inability of chemotherapy to eradicate refractory AML subclones. Refractory AML has been demonstrated to be hallmarked by alterations in mitochondrial metabolism. Recently, we discovered a multidrug resistant AML subtype characterized by low oxidative phosphorylation (OXPHOS) activity but paradoxically high mitochondrial membrane potential. This subtype was initially perplexing, as, in normal healthy mitochondria, high membrane potential typically tracks with high OXPHOS. However, experiments combining direct quantification of both OXPHOS flux and mitochondrial membrane potential revealed that mitochondria in refractory AML sustain polarization via a non-canonical mechanism typified by OXPHOS reversal. In this way, rather than synthesizing ATP, mitochondria in AML reverse the ATP synthase reaction to pump protons across the inner membrane, thus ensuring constitutive mitochondrial polarization. Because mitochondrial depolarization initiates apoptosis, we hypothesized that AML cells resist apoptosis by hydrolyzing ATP to sustain mitochondrial membrane potential. Consistent with the hypothesis, upon exposure of AML cells to the pro-apoptotic BLC-2 inhibitor venetoclax, AML cells hydrolyzed ATP to sustain mitochondrial polarization. To determine if mitochondrial membrane potential was actionable in AML, we leveraged a family of small molecules we discovered to induce AML mitochondrial depolarization. The shared ability of these compounds to induce depolarization derives from the fact that they are all positively charged, and thus are functionally classified as organic cations. Organic cations accumulate in the negatively charged mitochondrial matrix where they directly interfere with mitochondrial polarization. Across multiple AML cell lines, organic cation exposure synergized with venetoclax to induce cell death. Taken together, these data highlight mitochondrial polarization as an actionable vulnerability in AML and provide pre-clinical evidence in support of leveraging organic cations to treat AML.

POSTER 29.

Inhibition of Bcl-2 family members forces reversal of the ATP Synthase reaction

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Abstract: Strategies to target the mitochondria in cancer frequently involve inhibition of oxidative phosphorylation (i.e. OXPHOS). Because OXPHOS is functional across all mammalian mitochondria, there is potential for off-target toxicity in organs that rely on efficient ATP synthesis (e.g. heart, skeletal muscle, brain, kidneys). Therefore, therapeutic efficacy of treatments based on inhibiting OXPHOS is limited. To advance these efforts, we sought to identify mitochondrial phenotypes unique to malignant cells for the purpose of discovering cancer-specific drug targets. In this investigation, we assessed the functional role of ATP Synthase in a variety of healthy blood cells, blood cancer cells, and in addition, solid cancer cells. Of the 14 cancer cell lines used, 11 of them presented with significant reductions in OXPHOS capacity relative to respiratory capacity. Further investigation linked this phenotype to an intrinsic reversal of the ATP synthase reaction, despite an availability of carbon sources for oxidation. Finally, we demonstrated that inhibition of pro-survival Bcl-2 family members, Bcl-2 and Bcl-xL, forced reversal of the ATP synthase reaction via a loss of respiration. Collectively, these findings demonstrate a novel phenotype (i.e. intrinsic reversal of ATP Synthase) that is prevalent in a wide range of cancers, and highlight a novel role of Bcl-2 family proteins in mitigating reversal of the ATP synthase reaction.

POSTER 30.

Physiological deficiencies in respiratory complex I accelerate tumor outgrowth in colorectal adenocarcinoma

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Relative to matched normal tissue, recent large scale sequencing efforts indicate that colorectal cancer (CRC) tumors are specifically enriched in loss of function mutations in mitochondrial DNA (mtDNA); however, the significance and functional consequences of high mtDNA mutational burden in CRC remains unknown. Given that mtDNA encodes critical subunits in 4 out of the 5 complexes of the electron transport chain (ETC), high mtDNA mutational burden suggests that CRC incidence and/or pathogenesis is dependent on disruptions in mitochondrial respiration. On the contrary, impairing tumor mitochondrial respiration, via pharmacological ETC inhibitors or deletion of genes required for the function of the respiratory complexes, blunts tumor growth across many tumor types, including CRC. Together, these seemingly contradictory data sets highlight an intriguing paradox: *how do accumulated mtDNA mutations support CRC tumorigenesis, if mitochondrial oxidative metabolism is inherently required for tumors to grow?* Generating targeted and efficient CRC therapeutics is dependent on answering this question. In preliminary studies using purified human CRC mitochondria we confirmed that, relative to matched normal, CRC tumors have more mtDNA mutations and discovered that functional bioenergetic deficiencies exclusively localize to mitochondrial complex I, with 100% (12/12) of clinical CRC tumors displaying partial loss-of-function in complex I activity. To model human CRC bioenergetic deficiencies in the mouse, we reduced complex I activity by 50% in the colon using tissue-specific deletion of the complex I accessory subunit NDUFS4. Partial complex I inhibition increased both tumor number and size following CRC initiation with azoxymethane/dextran sodium sulfate (AOM/DSS) and induced a pronounced growth advantage in tumor-derived organoids. These results demonstrate that partial complex I loss of function provides a growth advantage sufficient to accelerate CRC outgrowth. Surprisingly, despite it's role as the initiating complex of the ETC, complex I deficient CRC tumors respired normally; although at the expense of increased matrix NADH/NAD^+. Additional bioenergetic analysis revealed that CRC tumors circumvent NADH/NAD^+ hyper-reduction to sustain mitochondrial oxidative metabolism by uncoupling respiration from ATP synthesis. Taken together, our findings indicate that CRC mitochondria exhibit unique metabolic rewiring initiated by primary deficiencies in respiratory complex I. Partial complex I loss-of-function provides a growth advantage that accelerates CRC outgrowth, potentially by sustaining chronic elevations in matrix NADH/NAD^+ and/or inducing respiratory uncoupling.

POSTER 31.

Regulation of mitochondrial metabolism by snoRNAs in atherosclerosis

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Atherosclerosis (athero) is a major cause of death of cardiovascular diseases. Reactive oxygen species (ROS) has emerged as a key contributor of athero. Excess ROS produced by macrophages (Mφs) and smooth muscle cells (SMCs) exacerbates inflammation and promotes foam cells formation. Previously, Dr. Holley and others has revealed a novel mechanism regulating ROS and oxidative stress, involving four small nucleolar RNAs (snoRNAs) that are intronically-encoded at the Ribosomal protein L13a (Rpl13a) locus: Snord32a, -33, -34, and -35a. Loss of these snoRNAs protects cells from metabolic oxidative stress. These snoRNAs canonically guide 2'-O-methylation (Nm) of rRNA, but we have reported that Snord32a also inhibits translation of the ROS enzyme PXDN by guiding Nm modification of Pxdn mRNA. We thus hypothesized that Rpl13a snoRNAs contribute to athero by modifying their potential RNA targets. To test this hypothesis, we generated Rpl13a snoRNA KO (snoKO) mice (lacking all 4 snoRNAs but without affecting RPL13A protein expression), on Apoe-/- background and high-fat diet. Our preliminary data shows that snoKO mice develop 50% less athero than wild-type (WT) mice. What's more, snoKO SMCs and Mφs have lower mitochondrial ROS levels than WT. Seahorse assays revealed an alleviated mitochondrial metabolism in snoKO SMCs compared with WT. We have also identified at least two mitochondrial genes regulated by Rpl13a snoRNAs: Cox4i2 and Ucp3. These findings suggested that snoRNAs play a critical role in atherosclerosis by regulating mitochondrial metabolism.